FACTORES DE RIESGO ASOCIADOS A LA SEROPREVALENCIA DE *NEOSPORA CANINUM* EN GANADO LECHERO DE PASTO, NARIÑO

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Trabajo de grado presentado como requisito parcial para optar al titulo de Medico Veterinario

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San Juan de Pasto, Diciembre de 2011

CONTENIDO

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Seroprevalence and risk factors associated to *Neospora caninum*in dairy cattle herds in Pasto, Colombia

Factores de riesgo asociados a la seroprevalencia de *Neospora caninum* en ganado lechero de Pasto, Nariño

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ABSTRACT

Objective. To determine the seroprevalence and risk factors associated to *Neosopora caninum* in non- vaccinated dairy cattle in the municipality of Pasto, Colombia. **Materials and methods**.Farms over2527meters over sea level were selected. A total of 238 serum samples were collected and analyzedusing theELISAtest to determine the seropositivity against *Neosopora caninum*. A questionnaire, which included variables, related to cattle, health and management measures were filled out in each herd. A multivariate analysis binary logistic regression was usedwith aconfidence intervalof 95% ($p<0.05$) using the programSPSS19®.**Results.**The estimated prevalence of exposure to *Neosopora caninum* showed a seropositivity of 76.9%. The risk factors in this region are residues of abortions, which are not buried but left outdoors (OR 3.81, 95% CI 1.5 to 9.6), feeding dogs with swilling (OR 15.44 IC 95% 1.94-123.22) and the used of bulls during direct mount (OR 19.68, 95% CI 2.34 to 165.52)**Conclusions.**The high prevalence of *Neospora caninum* and low level abortion in the herds in the municipality of Pasto shows the no existence of the disease in animals serologically positive, suggesting that at some point of its life were exposed to the agent. From the identified risk factors associated in this study we can provide recommendations for a control of reproductive diseases in the region.

Key words: abortion, fetal death, parasites, serology.

RESUMEN

Objetivo. Determinar la seropositividad de *Neospora caninum* en bovinos no vacunados contra el síndrome reproductivo y los factores de riesgo asociados a la presentación de esta enfermedad en los hatos lecheros del municipio de Pasto, Nariño. **Materiales y métodos.** Se seleccionaron fincas sobre 2527 msnm. Se analizaron muestras de suero de 238 vacas Holstein en producción mediante la prueba de ELISA. Se realizó un cuestionario relacionado con las variables sobre el ganado, medidas sanitarias y de manejo. Se realizó un análisis multivariado mediante regresión logística binaria con un Intervalo de Confianza del 95% ($p<0.05$) utilizando el programa SPSS19®. **Resultados.** La seropositividad para *Neospora caninum* fue de 76.9 %. Se determinó que los factores de riesgo en esta región son los residuos de abortos, que no se entierran y se dejan a la intemperie (OR 5.49; IC 95% 1.7-17.7), alimentar los perros con desperdicios (OR 15.44 IC 95% 1.94-123.22) y la monta directa (OR 14.62 IC 95% 1.55-137.53). **Conclusiones.** La elevada prevalencia de *Neospora caninum*y los niveles bajos de abortosen el municipio de Pasto no indica la enfermedad existente en los animales positivos serológicamente, pero sugiere es que en algún momento de su vida fueron expuestos al agente causal. A partir de los factores de riesgo identificados en el presente trabajo se pueden establecer recomendaciones para un control efectivo de enfermedades reproductivas presentes en la región.

Palabras clave: muerte fetal, aborto, serología, parásitos.

INTRODUCCION

Bovine neosporosis is a parasitic disease caused by the protozoan Neospora caninum and is considered one of the main causes of abortion in cattle worldwide (1). The abortions due to infection have been reported Nesopora a substantial economic loss in the livestock industry (2). The transmission from an infected cow to its offspring has been identified as the main route of infection (3) and is the most important way to maintain the infection in herds due to the elimination of the parasite in aborted fetuses. The dog has been identified as the definitive host, where the sexual stage of the protozoa takes place in the gut with the formation of oocysts, which are disposed with the feces and then ingested by cattle. In cows starts the phase with the formation of asexual tissue cysts and tachyzoites in the tissues of intermediate host or fetus. Dogs become infected after ingesting infected tissues such as

an aborted foetus or placenta tissue completing the epidemiological cycle with the formation of oocysts. (4,5). After the birth cattle infects due to contamination of drinking water or feed contaminated by dog or other carriers not yet identified (6,7). Abortions can occur at any time of gestation sporadically or in outbreaks (8), but has a larger display between 4 and 6 months gestation (6). It has been reported a higher prevalence in dairy cattle than beef because there are management factors that favor the spread of the disease (9).

In south American have been reported seroprevalence of N. caninum; in Argentina was shown a prevalence of 64.5% in cows with clinical history of abortion (10), Paraguay was established by ELISA seroprevalence of 29.8%, and identified as the major cause of abortions in cattle meat and milk (11), in Brazilian state of Parana found a prevalence of 15.1% in dairy Holstein breed (12), Aguascalientes Mexico found a seroprevalence of 57.5% in cows with a clinical history of abortion (13), in the northwestern United States found a seroprevalence of N. caninum 24% which increases in winter, times attributed to the high density of cattle (14). In Colombia in 2001 reported a 54.1% seroreactivity in 357 animals with a history of abortion (15). In Nariño there has been no epidemiological studies on patterns of *Neospora caninum*, therefore the objective of this study is to determine the prevalence and risk factors associated with the parasite.

MATERIALS AND METHODS

Study Site. The study was conducted in 10 dairy farms in the rural municipality of Pasto (Nariño-Colombia) location nearby the Galeras volcano, belonging to an ecosystem of lower mountain dry forest according to the Holdridge classification. With a 700 mm annual precipitation, average temperature of 13.3°C and humidity of 60% to 88%. These farms were extensively managed, cows appending most of the time on fenced pastures, with no physical separation between heifers and adult cows.

Cattle population. Farm prevalence levels were obtained by a cross-sectional study using a strategy of simple random sampling of animals from dairy farms in the municipality of Pasto as follows:

$$n = \frac{N * Z_2 * P * (1 - P)}{N * e_2 + Z_2 * P * (1 - P)}$$

Where:
Nis the number ofdairy cowsin the municipality
P: Expected prevalence
e: Acceptable Error(in this study10%)
Z: confidence level (95% = 0.05)

For the number of animals needed to sample for estimating the individual level prevalence we assumed an expected prevalence of30%, combined with an acceptableerror of 10%and confidence levelof 95%. 238 Holstein cows which had never been vaccinated against Neosporosis were selected. The cows had a moderate level of milk production (15 kg/cow/day), registrationof reproductive eventsand identificationof the animal.Among theinclusion criteriawere: breeding cows that have calved at least once withmore than 6 monthsremainingon the farms.

Variables. Epidemiological data were collected through a structure fill in questionnaire obtained by a direct interview with the cattle farmer. The variables included were group by topics for neosporosis: Management: Type of reproduction (natural or artificial insemination), synchronization and culling. Health; annual abortion, deworming and vaccination. Pasture management; organic fertilizer, manureas fertilizer. Origin of replacements cows; external, same farm or mixed. Biosecurity; Disposalof fetus andplacentas and water source.Presence of animals; Sheep, horses, pigs, cats and dogs.

Sample collection and serological examination. Blood samples (10 ml) were collected by puncture of the tail vessel using sterile tubes without anticoagulant (Vacutainer) which were subsequently transported and processed to separate the serum by centrifugation (1500 rpm / 5 minutes) and stored at -20°C until analysis in the clinical diagnostic laboratory veterinarian at the University of Nariño.

The presence of antibodies against *Neospora caninum* were tested using a commercial indirect enzyme-linked immunosorbent assay (ELISA) kits (Uppsala, Sweden, Svanova Biotech ®), following the manufacturer's specifications. The sensitivity and specificity of the test was 99% and 96% respectively. The plates were read at 450nm and the results were given in optical density values expressed in percentage of positivity for antibodies to *Neospora caninum*. The formula used was:

$$(pp)\%\ positivityvalues = \frac{sampleornegativecontrol(ODcorrected)}{positivecontrol(ODcorrected)} x100$$

The control values were in the following limits; the positive control optical density (OD) > 0.8 and OD negative control <0.15. Results with values ≥20% were considered positive and below negative.

Data analysis. The apparent prevalence of antibodies to *Neospora caninum* was estimated from the ratio of positive results to the total number of cattle examined. The association between seroprevalence and risk factors was quantified using a multivariate binary logistic regression with a confidence interval of 95%. The significance of the association was estimated by determining Odds Ratio (OR) of each factor with a P value <0.05. The goodness of fit was assessed with statistics Hosmer - Lemeshow. Calculations were performed using SPSS 19®.

RESULTS

Sampled herds were characterized by small size and a moderate level of milk production (15 kg/cow/day) that would correspond with traditional managed herds with low levels of specialization. Percentage of abortions in this study was of 7%. The medical records of the farms reported placental retention return to estrus after service, increased services per conception, interacting directly with the open days ranging from 140 ± 20 days. The farms only had records of vaccination against Mouth and foot disease and brucellosis. 183 cows were ELISA seropositive; the animal level prevalence for antibodies to *Neospora caninum* estimated at 76.9% (Table 1).

Table 1.. Prevalence of antibodies to *Nesopora caninum* in Holstein cows in milk in the municipality of Pasto, Nariño (Colombia).

Farm	Number of cows	Number of positive cows to Neospora	Number of negative cows to Neospora	Prevalence (%) Neospora
A	27	22	5	81.5
B	25	15	10	60.0
C	19	13	6	68.4
D	8	3	5	37.5
E	22	17	5	77.3
F	15	13	2	86.7
G	38	24	14	63.2
H	23	21	2	91.3
I	47	42	5	89.4
J	14	13	1	92.9
TOTAL	238	183	55	76.9

In nine farms the serpositiviyty was over a 60 %. The prevalence for *Neospora caninum* in the municipality of Pasto was of 76.9%. Three variables were associated with seropositivity to Neosporosis;residues of abortions, which are not buried but left outdoors, feeding dogs with swillingand the used of bulls during direct mount (Table 2).

Table 2.Risk factors associatedwith seropositivityof*Neospopra caninum*in lactating cowsonfarms in themunicipality ofPasto, Nariño.

Variable	[2]OR	[1]IC 95% Lower	Upper	[3] P-Value
Used of bulls during direct mount	14.62	1.55	137.53	0.012
Residues of abortions	5.49	1.7	17.7	0.003
Feeding dogs with swilling	15.44	1.94	123.22	0.006

[1] Confidence interval95%, [2]Odds ratio, [3]$P< 0.05$

Other variables showed a low association between the characteristics of the farm, management and the serologic results of cattle; presence of other animals (Sheep, horses, pigs, cats, and dogs), pasture management; organic fertilizer, manureas fertilizer and origin of replacements cows; external, same farm or mixed.

DISCUSSION

The seroprevalence of *Neopspora caninum* within the region is high compared to other parts of the country, as reported in Monteria with a seropositivity of 10.2% in cows with reproductive problems (16) and other work in Colombia by Zambrano et al with values of 54.1% of seroreactivity in 357 animals with a history of abortion, using the same diagnostic technique ELISA (15). In Antioquia the prevalence found in Holstein cattle was of 39.9% (17). Abortion is the most commonly reported event in cases of neosporosis (18), however in this work, the abortion rate was 7%. In the present study has questioned the association between other reproductive abnormalities and seropositivity to the agent. Most of these farms have reproductive problems at some point, the most common being the high range of open days which exceeds the estimated range and repetition of heats. It also states that the fetal mummification is a common event in cases of neosporosis described in cases of natural and experimental infections (19), in the present study only in one farm the veterinarian reported this situation.

In this study the presence of dogs is common on all farms, which cannot be determined statistically as a risk factor bacuse of the collinearity. Guimaraes et al (12) in Brazil, found no association the presence of dogs on farms with Neospora seropositivity, however other authors describe the presence of canine defecation in the pastures, the presence of stray dogs and other wild animals such as foxes a strong association with the presentation of N. caninum (20,21). In this study, dogs fed on waste and residues of abortions is a risk factor, since dogs when they eat contaminated material with tachyzoites enter in the cycle as definitive hosts (22).

The disposal of aborted material becomes a major risk factor in this study, contrary to those reported in Aguascalientes, Mexico (13) where no association was found between the provision of aborted fetuses and placental debris with seroprevalence. Foetus and / or stillbirths and poor provision of these on the farm makes an environment conducive to the proliferation of the parasite, contaminating pasture, water and facilities. Animals found within the premises that enter in the parasite cycle may contaminate because oocysts are environmentally resistant (23).

In this study we found that a common practice in the region is the use of direct mount, becoming a risk factor. Although farmers use artificial insemination, cows categorized as reproductive problem when repeat

heat, the bulls are allowed to mount. In experiments conducted in the U.S. show that venereal transmission is possible with a large number of tachyzoites (24). In this study, two of three bulls sampled were seroreactivity for antibodies to N. caninum. Benavides et al (25), consider that there is a strong association with the presentation of abortions the absence of drainage systems and septic tank in the department of Nariño, in the management of wastewater and storm water as this favors the maintenance of Neospora and leptospira, however on these farms was not a risk factor for the disease under study. Regarding the management of replacements, raised on farms or purchased was not risk factor associated with seropositivity of *Neospora caninum*. Farmers that breed their own replacements on farms, endemic neosporosis transmission is the main mechanism for maintaining the high prevalence (26).

In conclusion this study shows a high general and individual seroprevalence to *Neospora caninum* in dairy Holstein herds in the municipality of Pasto, Colombia. Due to the type of herd management carried out in this population, these results could indicate the no existence disease in animals serologically positive, but suggests that at some point in his life were exposed to the disease. The seroprevalence of *Neospora caninum*in the municipality of Pasto from 2001 to the present has not had greater variations in contrast to other regions of the country. Under these circumstances and according to this study, dairy health programmes should be directed at correct disposal of abortions and health control of other animals.

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